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## The Nature of the Ascus Wall: A Preliminary Study

CARL MALONE<sup>1</sup>

**SYNOPSIS:** The Nature of the Ascus Wall: A Preliminary Study. *Proc. Iowa Acad. Sci.*, 79(2):70-71, 1972. The ultrastructure of the ascus wall of five free-living and two lichenized Ascomycetes is briefly described. The unitunicate ascus is interpreted to be two layered, and the bitunicate ascus is interpreted to be four layered.

It is suggested that the terms "single layered" and "two layered" not be used synonymously with the terms "unitunicate" and "bitunicate" respectively.

**INDEX DESCRIPTORS:** Ascus, Ascus ultrastructure, Ascus wall

Different types of asci are characteristically associated with taxonomic groups of Ascomycetes (Luttrell, 1951, and Dennis, 1968). Unitunicate asci are generally associated with the Euascomycetes, while bitunicate asci are generally associated with Loculoascomycetes. However, few attempts have been made to integrate the lichen asci into the known ascus types (Dennis, 1968). Chadeaud (1942) has long illustrated unitunicate asci as two layered. The difference between the bitunicate and unitunicate ascus was that the two layers separated in the bitunicate ascus and did not separate in the unitunicate ascus. Reynolds (1971) studied the ultrastructure of the bitunicate ascus of *Limacinula theae* and reported the bitunicate ascus to be two layered. Moore (1962), Reeves (1967), and Greenhalgh and Griffiths (1970) illustrated the unitunicate ascus wall in their ultrastructural studies.

For this preliminary study, asci of five free-living and two lichenized Ascomycetes were selected in an attempt to evaluate differences in ascus wall ultrastructure. *Byssoschlamys nivea* was selected because of its unusual ascocarp and method of spore formation; *Eurotium ruber* for its evanescent, globose asci; *Chaetomium indicum* because its asci gelatinize by the time the spores mature; *Neocosmospora vasinfecta* to represent the Hypocreales; *Sporormia leporina* and *Arthopyrenia alba* to respectively represent the free-living and lichenized Loculoascomycetes of the Pleosporales; and *Caloplaca ulmorum* to represent a lichenized apothecial form with intensely bluing asci.

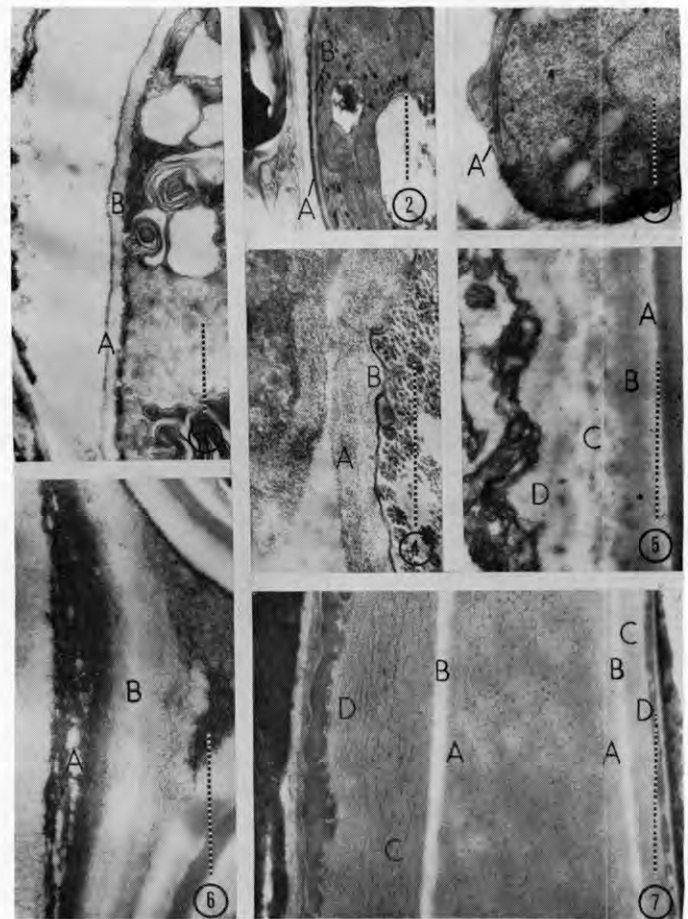
## MATERIALS AND METHODS

Specimens were fixed in 2% glutaraldehyde buffered to pH 7.5 in 0.1M phosphate buffer for 24 hours at 4°C. A graded ethyl alcohol series was used for dehydration, and propylene oxide was used as a solvent for the Epon embedment. Specimens remained in the pure Epon embedment for a week before polymerization was started. Sections were cut using glass knives and an LKB Ultratome III. Observations were recorded on a Hitachi HS-8 or an RCA EMU-3F electron microscope.

## RESULTS

The ascus walls of *Neocosmospora*, *Byssoschlamys*, *Chaetomium*, and *Eurotium* are relatively simple structurally (Fig-

ures 1, 2, 3, 4 respectively). There is a thick inner electron transparent layer with a thinner electron dense surface layer. Microfibrils may radiate outward from this surface layer. The



Figures 1-7. Line scales represent one micron. Figure 1. Ascus of *Neocosmospora vasinfecta* with outer dark (A) and inner transparent (B) layers. Figure 2. Ascus of *Byssoschlamys nivea* with outer dark (A) and inner transparent (B) layers. Figure 3. Gelatinizing ascus of *Chaetomium indicum* (A). Figure 4. Ascus of *Eurotium ruber* with outer dark (A) and inner transparent (B) layers. Figure 5 of *Arthopyrenia alba* with two layered outer (A and B) and two layered inner (C and D) walls. Figure 6. Ascus of *Caloplaca ulmorum* with outer dark (A) and inner transparent (B) layers. Figure 7. Ascus of *Sporormia leporina* with two layered outer (A and B) and two layered inner (C and D) walls.

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gelatinizing ascus wall of *Chaetomium*, just prior to spore maturation, is very thin with numerous microfibrils radiating from it (Figure 3).

The bitunicate ascus of *Arthopyrenia* and *Sporormia* (Figures 5, 7 respectively) is more complex than the unitunicate ascus. The exotunica and endotunica each consist of two different staining layers. The inner boundary of the endotunica with the ascus cytoplasm is irregular since small areas of the inner layer project into the cytoplasm. These projections are small in *Sporormia* and large in *Arthopyrenia*. The unexpanded endotunica is three times thicker than the expanded endotunica. Microfibrils of the endotunica are reoriented after the endotunica expands.

The ascus wall of the lichen *Caloplaca* consists of two layers (Figure 6). The inner electron transparent layer is nearly 0.75 microns thick, and the outer electron dense layer is nearly 0.5 microns thick. Microfibrils radiate from this outer layer. The boundary of the inner layer with the ascus cytoplasm is relatively smooth.

#### DISCUSSION

The structure of the unitunicate ascus as reported here is consistent with that reported for other unitunicate species (Rudolph and Geisy, 1966; Moore, 1962; Reeves, 1967; Greenhalgh and Griffiths, 1970). The *Caloplaca* ascus is interpreted to be unitunicate and differs from other unitunicate asci only in the amount of surface darkening and in the thickness of the two layers.

The bitunicate ascus is interpreted to consist of four different staining layers in contrast to the unitunicate ascus which has only two. By comparing expanded and unexpanded asci, the endotunica is interpreted to consist of two staining layers which decrease considerably in thickness when the endotunica expands. (Figure 7)

Previous studies and this study are consistent with Chade-

faud's belief that unitunicate asci are two layered. However, the bitunicate ascus as described here is not consistent with Chadeauf (1942) and Reynolds (1971). The bitunicate ascus is four layered and is more complex than the unitunicate ascus.

The terms "single layered" and "two layered" are often used synonymously with "unitunicate" and "bitunicate" respectively. As shown in this and previous studies, unitunicate and bitunicate asci both consist of more than one layer. To avoid unnecessary confusion, the term "layer" might best be restricted to demonstrable regions within a single wall.

In light of existing studies and this preliminary study, a survey of various ascus types should be made. Particular attention should be given to the fine structure of the ascus wall prior to spore formation, during spore maturation, and after spore dispersal. Some relatively simple histochemistry at the EM level would also be enlightening.

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